

CLAIMS

1. A method of producing scyllo-inositol comprising:
allowing a microorganism capable of converting myo-inositol into scyllo-inositol and belonging to the genus *Acetobacter* or *Burkholderia* to react with myo-inositol in a solution containing myo-inositol to produce and accumulate scyllo-inositol in the solution; and
collecting the scyllo-inositol from the solution.
2. The method according to claim 1, wherein the solution containing myo-inositol is a liquid medium containing myo-inositol, and the microorganism is allowed to react with myo-inositol by culturing the microorganism in the liquid medium.
3. The method according to claim 1, wherein cells obtained by culturing the microorganism is allowed to react with myo-inositol in the solution.
4. The method according to any one of claims 1 to 3, wherein the microorganism is a microorganism belonging to *Acetobacter cerevisiae*, *Acetobacter malorum*, or *Burkholderia andropogonis*.
5. The method according to any one of claims 1 to 3, wherein the microorganism is *Acetobacter* sp. AB10281 strain (FERM BP-10119) or a mutant strain thereof.
6. *Acetobacter* sp. AB10281 strain (FERM BP-10119) or a mutant strain thereof having an ability to convert myo-inositol into scyllo-inositol.
7. NAD⁺-independent myo-inositol 2-dehydrogenase having at least the following physiological properties:
 - (a) Action: catalyzing a reaction that deprives myo-inositol of electron to produce scyllo-inosose in the presence of an electron accepting substance
 - (b) Optimum pH: the activity is maximum at pH of 4.5 to 5.5
 - (c) Cofactor: containing 1 mol of heme iron per 1 mol of the enzyme
 - (d) Inhibitor: the activity of the enzyme is inhibited to 1% or lower by 1 mM of Sn²⁺ ion
 - (e) Subunit structure: a heteromer at least comprising proteins each having a molecular weight of 76 k Dalton or 46 k Dalton
 - (g) Substrate specificity: acting on D-chiro-inositol, muco-inositol, and myo-inositol to convert them into D-chiro-1-inosose, L-chiro-2-inosose, and scyllo-inosose, respectively, but not acting on allo-inositol, scyllo-inositol, L-chiro-inositol, and glucose
8. A method for producing myo-inositol 2-dehydrogenase, comprising:
culturing a microorganism which has an ability to produce NAD⁺-independent myo-inositol 2-dehydrogenase and belongs to the genus *Acetobacter*; and
separating and purifying the myo-inositol 2-dehydrogenase from the cells of the

cultured microorganism.

9. The method according to claim 8, wherein the microorganism is *Acetobacter* sp. AB10253 strain (FERM BP-10136).

10. A method for producing scyllo-inosose, comprising:

generating scyllo-inosose by allowing NAD^+ -independent myo-inositol 2-dehydrogenase to react with myo-inositol in a solution containing myo-inositol and an electron acceptor; and

separating and purifying the generated scyllo-inosose from the solution.

11. A method for producing scyllo-inositol, comprising:

generating scyllo-inosose by allowing NAD^+ -independent myo-inositol 2-dehydrogenase to react with myo-inositol in a solution containing myo-inositol and an electron acceptor;

generating scyllo-inositol by allowing the scyllo-inosose to react with a reducing agent; and

separating and purifying the scyllo-inositol.

12. A method for screening a microorganism for producing scyllo-inosose, comprising:

subjecting *Acetobacter* sp. AB10253 strain (FERM BP-10136) to a mutagenesis treatment to obtain mutant strains; and

selecting a strain from the mutant strains based on NAD^+ -independent myo-inositol 2-dehydrogenase activity.

13. A method for screening a microorganism for producing scyllo-inosose, comprising:

isolating microorganisms from a natural sample containing the microorganisms;

selecting a microorganism from the isolated microorganisms based on NAD^+ -independent myo-inositol 2-dehydrogenase activity.

14. A method for producing scyllo-inosose, comprising:

generating scyllo-inosose from myo-inositol by culturing the microorganism for producing scyllo-inosose obtained by the screening method according to claim 12 or 13 in a medium containing myo-inositol; and

separating and isolating the generated scyllo-inosose from the medium.

15. A method for producing scyllo-inositol, comprising:

generating scyllo-inosose from myo-inositol by culturing the microorganism for producing scyllo-inosose obtained by the screening method according to claim 12 or 13 in a medium containing myo-inositol;

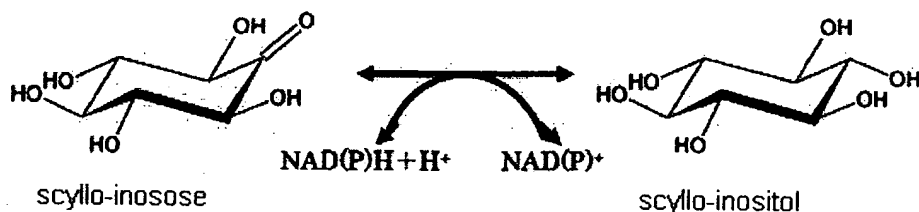
generating scyllo-inositol by allowing the scyllo-inosose to react with a reducing

agent; and

separating and isolating the generated scyllo-inositol from the medium.

16. A scyllo-inositol dehydrogenase having the following physiological properties:

Reaction: as shown in the following formula, catalyzing an oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reducing scyllo-inosose to scyllo-inositol in the presence of NADH or NADPH



17. The scyllo-inositol dehydrogenase according to claim 16, further having the following physiological properties:

(1) Molecular weight and association property: 38 to 46 k Dalton, forming a dimer or a trimer

(2) Coenzyme: requiring NAD^+ or NADP^+ , or NADH or NADPH as a coenzyme

(3) Activating heavy metals: activated in the presence of Co^{2+} ion

(4) Inhibiting heavy metals: inhibited in the presence of Sn^{2+} ion

(5) Optimum pH: having an activity at pH of 5 to 9

18. A protein represented by the following (A) or (B):

(A) A protein comprising an amino acid sequence of SEQ ID NO: 28, or

(B) A protein comprising an amino acid sequence of SEQ ID NO: 28, whereby one or plural of amino acids are substituted, deleted, inserted, and/or added, and catalyzing the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reducing scyllo-inosose into scyllo-inositol in the presence of NADH or NADPH.

19. A DNA encoding a protein represented by the following (A) or (B):

(A) A protein comprising an amino acid sequence of SEQ ID NO: 28, or

(B) A protein comprising an amino acid sequence of SEQ ID NO: 28, whereby one or plural of amino acids are substituted, deleted, inserted, and/or added, and catalyzing the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reducing scyllo-inosose into scyllo-inositol in the presence of NADH or NADPH.

20. A DNA represented by the following (a) or (b):

(a) A DNA comprising a coding region of the nucleotide sequence of SEQ ID NO: 27,

or

(b) A DNA which hybridizes under stringent conditions with a DNA having the

nucleotide sequence of SEQ ID NO: 27 or a nucleotide sequence complementary thereto, and encodes a protein that catalyzes the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reduces scyllo-inosose into scyllo-inositol.

21. A vector comprising the DNA according to claim 19 or 20.

22. A transformant microorganism comprising the DNA according to claim 19 or 20 or the vector according to claim 21.

23. The transformant microorganism according to claim 22, wherein a host to be transformed is *Escherichia coli*.

24. A method for producing scyllo-inositol dehydrogenase, comprising:
culturing the transformant microorganism according to claim 22 or 23; and
collecting scyllo-inositol dehydrogenase from the culture product thereof.

25. A method for producing scyllo-inositol dehydrogenase, comprising:
subjecting myo-inositol as a substrate to an oxidation conversion reaction into scyllo-inositol at pH 6.0 to 8.5 in the presence of NAD^+ or NADP^+ , in a solution which contains the scyllo-inositol dehydrogenase according to claim 16 and myo-inositol dehydrogenase (EC 1.1.1.18) which catalyzes a reaction of oxidizing myo-inositol to generate scyllo-inosose in the presence of NAD^+ or NADP^+ .

26. The method according to claim 25, wherein scyllo-inositol is added at 0.01 to 3% into the solution.

27. The method according to claim 25, wherein scyllo-inositol is added at 0.2 to 0.5% into the solution.

28. The method according to claim 25, wherein cobalt salt and/or magnesium salt is added at 0.01 to 5.0 mM into the solution.

29. The method according to claim 25, wherein cobalt salt and/or magnesium salt is added at 0.2 to 2.0 mM into the solution.

30. The method according to claim 25, wherein the concentration of myo-inositol in the solution is adjusted to 5 to 22%; and wherein the scyllo-inositol which is generated by the enzymatic reaction is crystallized in the reaction solution, and is separated as a crystal from the reaction system by filtration.

31. The method according to claim 25, wherein the scyllo-inositol dehydrogenase is a protein represented by the following (A) or (B):

(A) A protein comprising an amino acid sequence of SEQ ID NO: 28, or

(B) A protein comprising an amino acid sequence of SEQ ID NO: 28, whereby one or plural of amino acids are substituted, deleted, inserted, and/or an added, and catalyzing the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reducing scyllo-inosose into scyllo-inositol in the presence of NADH or NADPH.

32. The method according to claim 25, wherein the scyllo-inositol dehydrogenase is a protein encoded by the DNA represented by the following (a) or (b):

(a) A DNA comprising a coding region of the nucleotide sequence of SEQ ID NO: 27, or

(b) A DNA which hybridizes under stringent conditions with a DNA having a nucleotide sequence of SEQ ID NO: 27 or a nucleotide sequence complementary thereto, and encodes a protein which catalyzes the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reduces scyllo-inosose into scyllo-inositol.

33. The method according to claim 25, wherein the scyllo-inositol dehydrogenase is a protein represented by the following (C) or (D):

(C) A protein comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, or 14, or

(D) A protein comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, or 14, whereby one or plural of amino acids are substituted, deleted, inserted, and/or added, and catalyzing the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reducing scyllo-inosose into scyllo-inositol in the presence of NADH or NADPH.

34. The method according to claim 25, wherein the scyllo-inositol dehydrogenase is a protein encoded by the DNA represented by the following (c) or (d):

(c) A DNA comprising a coding region of the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, or

(d) A DNA which hybridizes under stringent conditions with a DNA having the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13 or a nucleotide sequence complementary thereto, and encodes a protein which catalyzes the oxidation-reduction reaction between scyllo-inositol and scyllo-inosose and stereospecifically reduces scyllo-inosose into scyllo-inositol.

35. A method for producing a purified scyllo-inositol, comprising:

a first step of forming a scyllo-inositol/boric acid complex by adding boric acid and a metal salt into a liquid mixture containing scyllo-inositol and neutral sugar other than scyllo-inositol in an amount two times or more larger than that of scyllo-inositol dissolved in the liquid mixture, and by adjusting the pH of the liquid mixture to 8.0 to 11.0;

a second step of separating the complex from the liquid mixture;

a third step of dissolving the separated complex into acid to cleave into scyllo-inositol and boric acid; and

a fourth step of isolating and purifying the scyllo-inositol from the acidic solution or acidic suspension obtained from the third step.

36. The method according to claim 35, wherein, in the first step, the amounts of the boric acid and metal salt to be added is not less than twice mol, and not more than three times of the scyllo-inositol dissolved in the liquid mixture.

37. The method according to claim 35, wherein, in the first step, pH of the liquid mixture is adjusted to 9.0 to 10.0.

38. The method according to claim 35, wherein the metal salt to be added is one or more kinds of metal salts selected from the group consisting of NaCl, NaHCO₃, Na₂CO₃, Na₂SO₄, NaHSO₄, NaH₂PO₄, Na₂HPO₄, Na₃PO₄, borax, KCl, KHCO₃, K₂CO₃, K₂SO₄, KHSO₄, KH₂PO₄, K₂HPO₄, K₃PO₄, MgCl₂, MgCO₃, and MgSO₄.

39. The method according to claim 35, wherein the liquid mixture containing the scyllo-inositol and the neutral sugar other than scyllo-inositol is a liquid mixture containing myo-inositol and scyllo-inositol obtained by reducing scyllo-inosose in a solution containing scyllo-inosose.

40. The method according to claim 35, wherein, in the third step, the solution obtained by dissolving the complex in acid is adjusted to an acidic solution of 0.1 N or higher; and, in the fourth step, the acidic solution is contacted with an strong acidic ion exchange resin, and with a strong basic ion exchange resin or a boric acid-selective adsorbing resin, and then scyllo-inositol is precipitated from the acidic solution.

41. The method according to claim 35, wherein, in the fourth step, scyllo-inositol is precipitated by adding an aqueous organic solvent to the acidic solution or acidic suspension.

42. The method according to claim 41, wherein the aqueous organic solvent is ethanol or methanol; and the ethanol is added in a volume 0.3 to 3 times larger, or the methanol is added in a volume 0.3 to 5 times larger, than that of the acidic solution or acidic suspension.

43. The method according to claim 41, wherein the aqueous organic solvent is ethanol or methanol; and the ethanol is added in a volume 0.6 to 1.5 times larger, or the methanol is added in a volume 0.9 to 2 times larger, than that of the acidic solution or the acidic suspension.

44. A method of producing scyllo-inositol, comprising:

a first step of obtaining a liquid mixture containing myo-inositol and scyllo-inositol by reducing scyllo-inosose using a metal salt of boron hydride in a solution containing scyllo-inosose;

a second step of dissolving a scyllo-inositol/boric acid complex in the liquid mixture by adding an acid to the liquid mixture and adjusting the solution to be an acidic solution of 0.01 N or more; and

a third step of precipitating only scyllo-inositol by adding an aqueous organic solvent

to the acidic solution in an amount such that the myo-inositol is not precipitated.

45. The method according to claim 44, wherein, in the third step, the aqueous organic solvent to be added is ethanol, methanol, or 1-propanol; and the ethanol is added in a volume 0.2 to 0.4 times larger, the methanol is added in a volume 0.2 to 0.8 times larger, or the 1-propanol is added in a volume 0.2 to 0.4 times larger, than that of the acidic solution.

46. The method according to claim 44, wherein, in the third step, the aqueous organic solvent to be added is ethanol, methanol, or 1-propanol; and the ethanol is added in a volume 0.35 to 0.45 times larger, the methanol is added in a volume 0.45 to 0.55 times larger, or the 1-propanol is added in a volume 0.35 to 0.45 times larger, than that of the acidic solution.